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(71) Applicant: MATSUSHITA ELECTRIC
 INDUSTRIAL CO., LTD.
 1006, Oaza Kadoma
 Kadoma-shi, Osaka-fu, 571(JP)
 Applicant: KYOTO DAIICHI KAGAKU CO., LTD.
 57 Nishiaketa-cho Higashikujo Minami-ku
 Kyoto-shi Kyoto-fu(JP)

(72) Inventor: Nankai, Shiro
 4-50-12, Nasuzukuri
 Hirakata-shi, Osaka-fu(JP)
 Inventor: Kawaguri, Mariko
 202, 1-12-1, Dainichi-cho
 Moriguchi-shi, Osaka-fu(JP)
 Inventor: Yoshioka, Toshihiko
 4-15-11-302, Shinmori, Asahi-ku
 Osaka-shi, Osaka-fu(JP)

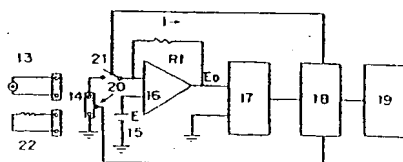
Inventor: Tsutsumi, Haruhiro
 1833-1, Tanokubo, Shigenobu-cho
 Onsen-gun, Ehime-ken(JP)
 Inventor: Terao, Kyoza
 1007-10, Kubota-cho, Kume
 Matsuyama-shi, Ehime-ken(JP)
 Inventor: Tanimoto, Naoki
 1058-15, Shitsukawa, Shigenobu-cho
 Onsen-gun, Ehime-ken(JP)
 Inventor: Yoshioka, Masahiro
 2-11-4, Higashiyosumi-cho
 Takatsuki-shi Osaka-fu(JP)
 Inventor: Hyodo, Hiroshi
 1640-8, Minamigasa-cho
 Kusatsu-shi, Shiga-ken(JP)
 Inventor: Uchigaki, Takatoshi
 5, Higashitsukurimichi, Koaza Kamikoma,
 Oaza
 Yamashiro-cho, Souraku-gun, Kyoto-fu(JP)

(74) Representative: Selting, Günther, Dipl.-Ing. et
 al
 Patentanwälte von Kreisler, Selting, Werner
 Deichmannhaus am Hauptbahnhof
 W-5000 Köln 1(DE)

(54) Quantitative analysis method and its system using a disposable sensor.

(57) A sensor 13 is inserted into a connector 14. A constant voltage required to obtain a response current is applied across the connector 14 by a voltage applying source 15 at timings required. A response current of the sensor 13 inserted into the connector 14 is converted into a voltage by a current-to-voltage converter 16, and the amount thereof is determined by a microcomputer, the analysis results being displayed onto a display unit.

Fig. 4



BACKGROUND OF THE INVENTION

1. Field of the Invention

5 The present invention relates to a quantitative analyzer for measuring such as a glucose level of biological fluid, particularly body fluid.

2. Description of the Prior Art

10 Various biosensors utilizing a specific catalytic action possessed by enzymes have recently been developed and applied, in particular, to clinical field. Development of biosensors having an ability of providing rapid and yet precise analytical results has long been desired in view of increasing number of samples and increasing number of items to be tested.

Diabetes mellitus is a disease from which the patient can not be completely recovered. However, the patient can live a normal life by keeping a concentration of glucose in blood at a normal level. Accordingly, constant retention of the normal glucose level is essential as a treatment of diabetes mellitus. The retention of the normal glucose level may be easily carried out on inpatients under physician's observation.

However, outpatients must conduct self-management in order to keep their blood glucose at a constant normal level. Such self-management includes dietary therapy, ergotherapy, and drug therapy, and the patients usually conduct the self-management on the above-noted two or more items under physician's directions. It is reported that when patients can check by themselves if their blood glucose level analytical results of glucose level in blood is within normal range or not, the self-management can be more effective.

In the treatment of insulin-dependent diabetes mellitus (IDDM), normal blood glucose level is maintained through repeated insulin-injections effected by patients themselves. However, the blood glucose level varies rapidly and considerably depending on caloric intake, dietary time, and injection time, and therefore, it is essential that the patients conduct the measurement of the glucose level by themselves.

Under such circumstances, various portable measurement systems have long been commercially available, which enable diabetes patients to conduct the glucose level measurement by themselves. Blood glucose level is generally determined using such a conventional measurement system in the following manner: whole blood which has been taken from a fingertip or ear lobe using a needle is contacted a test paper containing an enzyme specifically reacting with glucose and a color-producing reagent which develops color based on oxidation-reduction reaction; thereby the reagent and blood glucose react together and produce color, thickness of which is measured using an exclusive mini-reflectometer analyser attached to the system; the blood glucose level is determined on the basis of the calibration curve previously prepared and memorized in the analyzer.

However, it has been found that the blood glucose level determined according to the above systems varies greatly depending on patients' manipulation for measurement. Accordingly, Diabetes Associations in many countries have counseled the improvement of the measurement systems. The most important factor causing the above-noted variation of test results is associated with the manipulation needed for removing excessive blood from the test paper after a predetermined time. The removal of excessive blood is usually conducted through wiping with absorbent cotton, removing with a filter paper or rinsing with water, and such procedures bring about test errors in the following manner.

(i) Remaining blood on the test paper due to incomplete removal gives greater value than the real.

(ii) Excessive wiping or rinsing damages the test paper or washes out colored reagent, which gives smaller value than the real.

(iii) Inadequate manipulation which brings about shortage of reaction time causes insufficient coloration of the reagent, and mistimed manipulation makes it impossible to completely remove blood because of blood clotting or drying, thereby erroneous test results are obtained.

Moreover, when blood is contacted the test paper, the command (key input) of the timing for starting the measurement should be effected within an elapse of mistiming from ± 2 to 3 seconds. In actual cases (of some patients), however, this mistiming may be 30 seconds to one minute, which can be another factor for the variation of test results, causing less reliability of measured values.

In the last few years there has been commercially available a new measurement system (manufactured by Medisense Inc., commodity name: Exactech) which has solved a main part of the above problems. This system is a pen type system which displays measuring results 30 seconds after its measurement start switch is pressed the moment blood is fed onto its test electrode chip. The system has obviated the need of removing blood and the factors for considerable test errors.

Diabetics, in some cases, have poor blood circulation and are therefore susceptible to infectious

disease. This means that a slight wound on their hands or feet may cause suppuration, thus requiring the diabetics to keep themselves clean. Accordingly, equipment and a sensor for collecting blood in the measurement of blood glucose level are preferably provided in disposable form in view of hygienic control rather than used a plurality of times after they are sterilized and stored. This will ensure safety in hygiene and alleviate patients' burden.

As a method that allows a sensor to be disposable, a biosensor has already been proposed which is disclosed in the Japanese Patent Laid Open 61-294351. This biosensor, as shown in Fig. 1, is so constructed that electrode systems 136(136'), 137(137'), and 138(138') made of carbon or the like are formed on an insulating substrate 135 by a method of screen printing or the like, an insulating layer 139 is provided thereon, the electrode systems are covered with a porous body 141 carrying oxidoreductase and electron acceptors, and all these are integrated with a retaining frame 140 and a cover 142. When a sample liquid is dropped onto the porous body, the oxidoreductase and electron acceptors carried by the porous body are dissolved in the sample liquid, causing a reaction to proceed between the enzyme and substrate in the liquid and the electron acceptors to be reduced. After completion of the reaction, the reduced electron acceptors are electrochemically oxidized, and the resulting value of oxidation current is used to determine the concentration of substrate in the sample liquid.

However, in the Exactech, it is necessary to press the measurement start switch, which causes a defect that a considerable extent of mistiming in the measurement cannot be prevented. Moreover, its analyzer, being of pen type, makes its switch formed into one. As a result, since the calibration and adjustment of the analyzer must be carried out using this switch, the key operation involved has been made more complex unexpectedly. Also, since blood is placed onto the test electrode chips tipped by the pen and measurement is conducted without wiping the blood off, the patient is required to keep holding the analyzer during measurement so that the blood will not spill out. The system has therefore been inconvenient to use for the patients.

As described heretofore, since the self-management measurement system of blood glucose level conventionally available requires patients to conduct the command of starting measurement by themselves, it has been accompanied by such a defect that correct test results cannot be obtained depending on patients' manipulation. Moreover, complex key operation has been involved in operation for the calibration and test of the analyzer.

Conventional disposable systems, on the other hand, have been accompanied by such problems that test results may vary or that patients are required to distinguish whether a sensor has already been used or not.

SUMMARY OF THE INVENTION

The present invention has been achieved in view of the foregoing problems, and therefore its essential object is to provide a system and method in which the command of starting measurement can automatically be effected using a sensor having a capillary-shaped portion that obviates the need of removing excessive blood for self-measurement of blood glucose level, and in which calibration and test of the analyzer can be done without key operation.

Another important object of the present invention is to provide such a measurement system further capable of minimizing the variation of test results.

To accomplish these and other objects, the inventors have made every effort to find "a system and method in which the command of starting measurement can automatically be effected using a sensor having a capillary-shaped portion that obviates the need of removing excessive blood for self-measurement of blood glucose level, the variation of test results can be minimized, and in which calibration and test of the analyzer can be done without key operation".

Now the present invention will be described in detail.

The system of the present invention is used as a set with an exclusive sensor. The exclusive sensor is a "disposable electrode by the amperometric method," while the system is an "amperometric analyzer which displays the concentration of glucose calculated using a calibration curve from a measured current value."

In use of the system, with the sensor fitted into a sensor holder of the analyzer, the value of resistance at the electrode is infinity while blood is not supplied. Accordingly, the analyzer distinguishes that the sensor has been fitted into the holder, awaiting blood to be supplied.

When blood is supplied, the resistance value abruptly lowers. The sensor detects this lowering of the resistance value, and distinguishes that blood has been supplied, making the timer of the analyzer start. After a specified time, a constant voltage is applied to the sensor, and the resulting current is measured and

converted into a glucose level using a previously set calibration curve, the converted result being displayed as a measured value.

For adjustment of the analyzer, when a resistive chip (adjustment chip) having a sensor-like shape with a constant resistance value is fitted into the holder of the analyzer, it shows the constant resistance value initially. Accordingly, the analyzer distinguishes that it is not the sensor but an adjustment chip, preparing for the adjustment of the analyzer. Adjustment chips include an adjustment mode switching chip, an instrumental error compensating chip, a calibration chip, a test chip, and a unit switching chip.

The instrumental error compensation for the analyzer is carried out in the following manner.

When the adjustment mode switching chip is fitted into the holder of the analyzer, the sensor initially shows a constant low resistance value within a range assigned to the adjustment mode switching chip. From this fact, the analyzer distinguishes that it is the instrumental error compensating chip, switching the analyzer to the instrumental error compensation mode.

After adjustment of applied voltage, one of two types of compensating chips having predetermined different resistance values (R_L , R_H) is fitted into the holder of the analyzer, and the resulting measured value (R_1) is stored in the memory. Then, the other compensating chip is fitted into the holder and the resulting measured value (R_2) is stored in the memory; thereafter, a subsequent measured value R_n is compensated as a resistance value R according to the following Scheme 1:

$$R = \frac{R_H - R_L}{R_2 - R_1} \cdot R_n + \frac{R_L R_2 - R_H R_1}{R_2 - R_1} \quad (1)$$

For calibration of the analyzer, when the calibration chip is fitted into the holder of the analyzer, the sensor initially shows a constant resistance value within a range assigned to the calibration chip. From this fact, the analyzer distinguishes that it is the calibration chip, judging the type of calibration curve from the resulting resistance value. Whereas a plurality of types of calibration curves are stored in the analyzer, one type of calibration curve selected thereamong by the calibration chip is set and this is all of the calibration. Since the calibration curve differs depending on the production lot of sensors, sensors are supplied with calibration chips corresponding to each lot.

For test of the analyzer, when a test chip is fitted into the holder of the analyzer, the sensor initially shows a constant resistance value within a range assigned to the test chip. From this fact, the analyzer distinguishes that it is the test chip, displaying the resistance value as converted into the glucose level. The operator then distinguishes whether any abnormality exists in the analyzer according thereto. It may also be arranged that a normal range is previously stored in the analyzer so that existence of any abnormality will be displayed.

For switching of unit in the analyzer, when the unit switching chip is fitted into the holder of the analyzer, the sensor initially shows a constant resistance value within a range assigned to the unit switching chip. From this fact, the analyzer distinguishes that it is the unit switching chip, setting a unit corresponding to the resistance value.

When a used sensor is fitted into the holder of the analyzer, the sensor initially shows a low resistance value because the sensor is made wet by a blood sample, and moreover the value of current flowing through the sensor will vary with the resistance value gradually varying on account of polarization after a voltage is applied. Accordingly, the analyzer distinguishes that it is a used sensor on the basis of the elapsed stability of the current value (resistance value), displaying the fact on its display unit.

Further, the analyzer automatically detects that the sensor has been fitted in position into the reacting state, and interrupts the source of the reaction voltage or the like until the reaction is stabilized. This enables battery consumption to be suppressed.

According to the present invention, since the reaction voltage is applied after the reaction state is stabilized, variation of test results is minimized.

Furthermore, under the condition of high humidity, some sensors (for example, if its porous body 141 (see Fig. 1) is made of any hygroscopic material) are likely made wet due to humidification even though unused. The sensor thus initially shows a low resistance value, which further gradually varies on account of polarization after a voltage is applied. Due to this, the sensor may be mis-decided to be a used sensor. To prevent this, the above-noted disposable sensor is further provided with an electrode for detection of liquid junction so that the so-constructed sensor (see Fig. 6) will show a low resistance value when fitted into the sensor holder of the analyzer having such a circuit as shown in Fig. 5, and that it checks whether or not any liquid junction exists at the liquid junction electrode when the resistance value gradually varies, where if any

liquid junction exists, it distinguishes that a used sensor has been fitted, while if not, an unused sensor has been fitted, the sensor awaiting blood for measurement to be supplied.

A contact of the analyzer with the electrode for detection of liquid junction may also be used as the above-mentioned adjustment chip and test chip.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is an exploded perspective view showing an example of the sensor of a conventional measurement system;

Fig. 2 is a perspective view of an embodiment of a measurement system according to the present invention;

Fig. 3 is a perspective view of a sensor used in the measurement system in Fig. 2;

Fig. 4 is a block diagram showing an embodiment of a control unit used in the measurement system in Fig. 2;

Fig. 5 is a block diagram showing another embodiment of the control unit used in the measurement system in Fig. 2;

Fig. 6 is an exploded perspective view showing another example of the sensor used in the measurement system in Fig. 2;

Fig. 7 is an exploded perspective view showing a further example of the sensor used in the measurement system of the present invention;

Fig. 8 is a perspective view in which the sensor in Fig. 7 is assembled; and

Fig. 9 is a block diagram of a control unit used in combination with the sensor in Fig. 7.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A first embodiment of the present invention will be described below with reference to Figs. 2 to 6.

Fig. 2 shows an example of a system according to the present invention. Fig. 2 shows an example of a sensor to be used in combination with the system of the present invention.

Primary constituents contained in the reagent layer of the sensor are oxidoreductase which is specific for an objective substance in biological body fluid and a redox compound that makes an electron carrier of the enzyme.

As an example, the reaction measurement principle is described below in the case of measuring glucose level.

Glucose oxidase (hereinafter referred to as GOD) is used as an oxidase and potassium ferrocyanide is used as a mediator. When a test sample containing glucose is provided and contacted the sensor, an enzyme reaction occurs between the mediator and the glucose in the presence of GOD as shown in Scheme 2, whereby potassium ferrocyanide is produced in an amount corresponding to the glucose level. Then after an elapse of a specified time, a constant voltage is applied across a lead 8 of the sensor through the circuit used in the present invention. Since the oxidation current obtained therefrom is proportional to the concentration of potassium ferrocyanide produced by the enzyme reaction, i.e. glucose level, the glucose level in the subject body can be determined by measuring the response current.

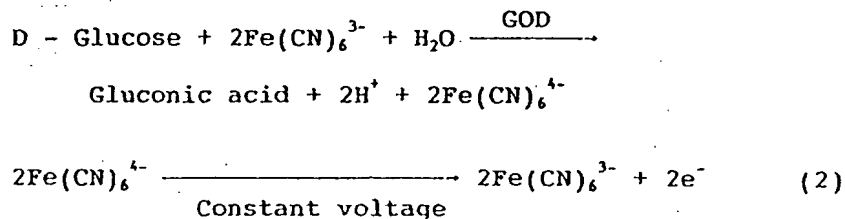


Fig. 4 shows a preferred embodiment of the present invention.

Referring to Fig. 4, the operation of the invention is now explained. First, a sensor 13 is inserted into a connector 14. When the insertion of the sensor 13 is detected by an electrode insertion detector switch 20, a switch 21 is closed so that a constant voltage required to obtain a response current is applied across the terminals of the connector 14 by a battery 15 serving as an applied voltage source. The response current of the sensor 13 inserted into the connector 14 is converted into a voltage by a current-to-voltage converter

16, and further inputted into an A/D converter 17.

A microcomputer 18 receives and reads an output signal from the A/D converter 17 and calculates glucose concentration. The sensor 13, enzyme electrode as it is, can be considered to be a type of resistor. For example, if the resistance value of the sensor 13 is R_s , the amplification resistance of the current-to-voltage converter 16 is R_f , and the applied voltage is E , then the output voltage E_o of the current-to-voltage converter 16 can be determined by the following calculation:

$$E_o = E + i \times R_f = E + (E/R_s) \times R_f$$

Without any sample supplied, since the resistance value R_s of the sensor 13 is extremely high and nearly infinity, the resulting current value i is accordingly extremely low, leading to that the output voltage E_o of the current-to-voltage converter 16 becomes nearly equal to E ($E_o \approx E$).

On the other hand, with a sample supplied to the sensor 13, since the resistance value R_s of the sensor 13 abruptly lowers with the value of E_o abruptly increasing conversely, the subject body can be sucked and detected by continuously monitoring the output voltage E_o of the current-to-voltage converter 16.

As a result, the measuring timer is automatically started by distinguishing the variation of the output voltage E_o of the current-to-voltage converter 16 with the aid of the A/D converter 17 using the microcomputer 18. With this operation, the switch 21 is simultaneously opened and, after an elapse of a specified time, closed, thereby allowing a measuring result to be obtained.

In order to adjust the analyzer, there is used an adjustment chip 22 having a shape similar to that of the sensor 13 and having a very small constant resistance value which is not to be compared with that of a new sensor (equal to infinity). Since the adjustment chip 22 initially shows a stable, constant voltage when measured, the microcomputer 18 can identify the adjustment chip 22, which is of various types, from the amount of the voltage.

Adjustment chips 22 include ones for uses of adjustment mode switching, instrumental error compensating, calibration, test, unit switching, and the like. When the chip is distinguished to be an adjustment mode switching chip, the analyzer is switched into the adjustment mode, the resistance value of the instrumental error compensating chip is stored, and measured values obtained thereafter are compensated. Normally, the adjustment mode switching chip is used when the analyzer is manufactured or remedied. For example, when the chip is distinguished to be a calibration chip, the microcomputer 18 automatically identifies and selects a calibration curve depending on the resistance value (voltage value) out of a plurality of calibration curves previously stored in the analyzer.

When the chip is distinguished to be a test chip, the microcomputer 18 converts the voltage value into a concentration and displays the result onto a display 2, allowing it to be judged from the amount of the concentration value whether any abnormality in the equipment exists or not.

When the chip is distinguished to be a unit switching chip, the microcomputer 18 changes and converts the concentration value into each concentration unit (for instance, mg/dl or mmol/L), then displaying it.

Table 1 shows a case of distinguishing calibration chips.

No. of calibration chip	Resistance value (K Ω)	No. of calibration curve
0	27	F - 0
1	30	F - 1
2	33	F - 2
3	36	F - 3
4	39	F - 4
5	43	F - 5
6	47	F - 6
7	51	F - 7
8	56	F - 8
9	62	F - 9

Also, the terminal of the connector can be increased in number in such an arrangement as shown in Fig. 5, so that a calibration chip or test chip can be inserted into a terminal other than that into which the sensor 13 is inserted.

However, it is possible that if the identification of calibration chips and test chips is done merely

depending on the amount of the resistance value, the chip may be mis-distinguished to be a calibration chip or test chip even when a used sensor is mis-inserted. This is caused by the fact that the resistance value of a used sensor is so low that it may be of the same level as those of the calibration and test chips.

To prevent this misidentification, the following method is adopted: Voltage value E_{01} is measured at the time point when power supply is turned ON with any electrode inserted into a connector of the system, and the voltage value E_{02} is measured once more after the succeeding several seconds. The resulting rate of voltage change ΔE is calculated and if it shows a change in voltage above a specified level, the chip is distinguished to be a used sensor, which is displayed on the display unit. Otherwise, the chip is distinguished to be a calibration chip or a test chip.

$$\Delta E = \left| \frac{E_{01} - E_{02}}{E_{01}} \right| \quad (3)$$

If the sensor, even though unused, has a property showing behavior similar to that of a used sensor due to humidification under high humidity condition, electrodes 9a, 9b for detection of liquid junction are provided in combination with the sensor, as shown in Fig. 6. When the above-noted used sensor is subjected to discrimination using both this sensor provided with electrodes for detection of liquid junction and the circuit shown in Fig. 5, it is distinguished that if the resistance value between the electrodes for detection of liquid junction 9a and 9b is infinity, there is no liquid junction, with such a decision made by the electrodes inserted into the connector that the sensor is an unused one, while if the resistance value between the electrodes for detection of liquid junction is low, the sensor is a used one. Meanwhile, the A/D converter 17 is used in combination by turning ON and OFF the switches 21, 21' through the microcomputer 18.

Fig. 7 is a detailed exploded perspective view of the sensor of the measurement system, which is a second embodiment of the present invention, and Fig. 8 is an outline perspective view of the same.

On a substrate plate 31 there are provided counter electrode 34 and a measuring electrode 35, leads 33, 32 connected thereto, and an insulating layer 36. Also, although not shown, there is formed a reaction layer containing an enzyme and a mediator so as to cover the counter electrode and measuring electrode. On the substrate plate 31 there is fixed a cover 39 with a spacer 37 interposed therebetween. Numeral 38 denotes a sample supply hole, through which a sample liquid, i.e. a sample is introduced onto the counter electrode 34 and measuring electrode 35 by capillarity phenomenon. Reference numeral 40 denotes an air hole. In order not to mistake the front and back of the sensor, there is provided an inverse-insertion preventing protrusion 41 so as to protrude from one side end of the sensor, whereby the sensor correctly directed up and down will properly be set with the inverse-insertion preventing protrusion passing through a counter gap of a connector 51, while the sensor, inversely set, will not be inserted into the connector 51 with an obstacle of the inverse-insertion preventing protrusion 41.

Fig. 9 is a block diagram of the control unit of a measurement system embodying the present invention.

First of all, the whole system is thrown into the standby state, starting up a CPU 50.

When the sensor 30 is inserted into the connector 51 of the main unit of the system, a detector circuit 52 detects the insertion of the sensor, turning on a current-to-voltage converter 53, an A/D converter 54, a temperature sensor 55, and other components through the CPU 50.

Next, when a sample liquid is supplied to the sensor so as to short-circuit the measuring electrode 35 and the counter electrode 34 with each other, the resistance value will vary to a great extent. The variation is distinguished by the CPU 50 through the A/D converter 54, turning off the current-to-voltage converter 53 with the result that no reaction voltage is supplied. Then, the reaction between the enzyme and sample liquid is allowed to proceed for approximately 55 seconds. During this period, the countdown state is displayed on an LCD display 56. Thereafter, a reaction voltage is applied for approximately 5 seconds, and the current is measured. This measured value is also displayed on the LCD display 56.

The voltage of a battery 57 is checked by the CPU 50 through a battery checker 58 for each one sequence of measurement, so that the voltage, if lower than a specified level, will be displayed onto the LCD display 56. A buzzer indicated by numeral 59 notifies that the sensor 30 has been inserted. An oscillator 60 generates pulses for clocking the operation of the system.

A memory 61 for storing compensation values for each system serves to compensate the variation among systems. Reference numeral 62 denotes a voltage regulator circuit. A circuit 63 serves to set a reaction voltage to be applied to the sensor. A circuit 64 serves to reset the CPU when, for example, measurement is stopped on its way or a battery is changed. A circuit 65 is a gain control circuit.

Although in the above-described embodiments the measurement system is normally in the standby state so that the actions such as applying a reaction voltage are not started until the sensor is inserted into the system main unit, thereby minimizing the number of parts of the system, the present invention is not limited to such an arrangement and allows another such that a standby switch is provided separately.

5 As described heretofore, according to the present invention, the introduction of samples can automatically be detected and, further, stable measurement with less variation of its results can be realized.

Claims

- 10 1. In a quantitative analysis system for measuring a specific component in biological body fluid by an amperometric method using a disposable sensor, a quantitative analysis method comprising the step of:
automatically distinguishing whether a chip mounted onto a sensor mounting portion of said system is a sensor for measuring a test sample, or an adjustment chip made up of a fixed resistor, or otherwise
15 any other else, depending on value of current flowing through the system when said chip is mounted thereon.
2. A quantitative analysis system for measuring a specific component in biological body fluid by an amperometric method using a disposable sensor, comprising:
20 means for automatically distinguishing whether a chip mounted onto a sensor mounting portion of said system is a sensor for measuring a test sample, or an adjustment chip made up of a fixed resistor, or otherwise any other else, depending on value of current flowing through the system when said chip is mounted thereon.
- 25 3. In a quantitative analysis system for measuring a specific component in biological body fluid by an amperometric method using a disposable sensor, a quantitative analysis method comprising the step of:
automatically distinguishing whether a sensor mounted onto a sensor mounting portion of said
30 system is a used one or not depending on stability of current value of said sensor.
4. A quantitative analysis method as claimed in claim 3, wherein said disposable sensor is provided with an electrode for detecting liquid junction, output of which electrode is used.
5. A quantitative analysis system for measuring a specific component in biological body fluid by an
35 amperometric method using a disposable sensor, comprising:
means for automatically distinguishing whether a sensor mounted onto a sensor mounting portion of said system is a used one or not depending on stability of current value of said sensor.
6. A quantitative analysis system as claimed in claim 5, wherein said disposable sensor is provided with
40 an electrode for detecting liquid junction, output of which electrode is used.
7. A quantitative analysis system which comprises:
means for applying a reaction voltage to a sensor;
means for detecting that a sample liquid is supplied to said sensor;
45 means for interrupting the reaction voltage on the basis of output of said detection means;
means for reapplying said reaction voltage to said sensor after said interruption means has operated for a specified time period;
means for detecting a reaction state of said sensor due to the reapplication thereof; and
50 means for displaying the reaction state.
8. A quantitative analysis system as claimed in claim 7, the system further comprising means for detecting that said sensor has been mounted to the system, wherein said reaction voltage application means is driven by the detection thereof.
- 55 9. A quantitative analysis system as claimed in claim 7 or 8, wherein said reaction voltage application means is a current-to-voltage converter, and said reaction state detection means makes a decision with a CPU on the basis of output of said converter adapted to analog-to-digital convert output of said current-to-voltage converter.

Fig. 1

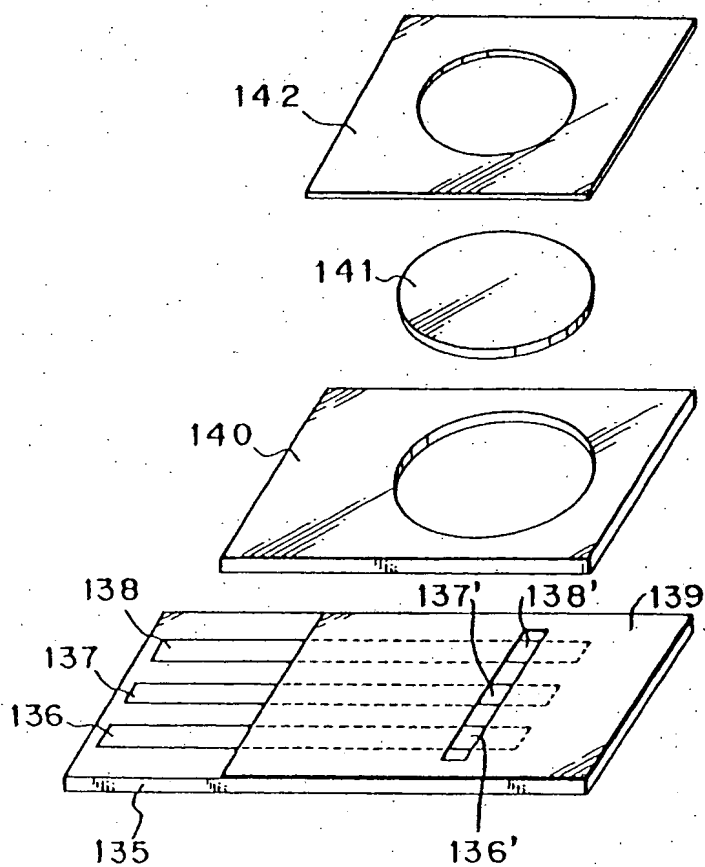


Fig. 2

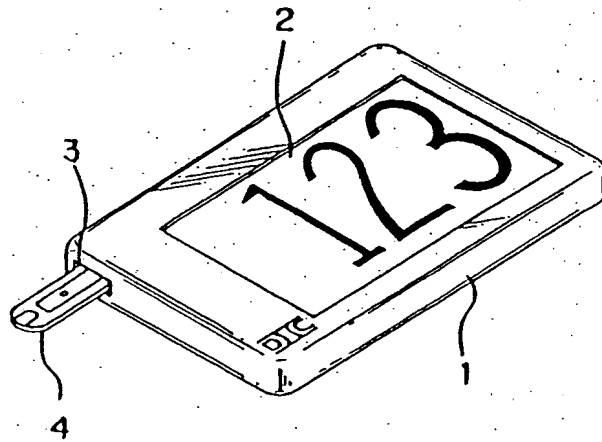


Fig. 3

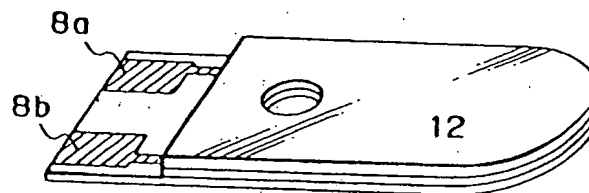


Fig. 4

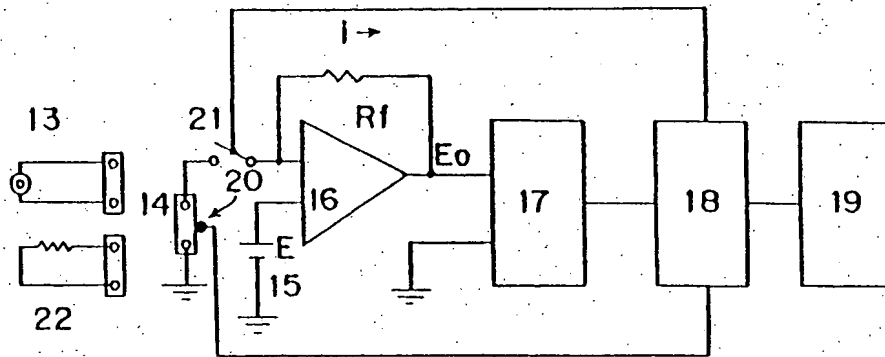


Fig. 5

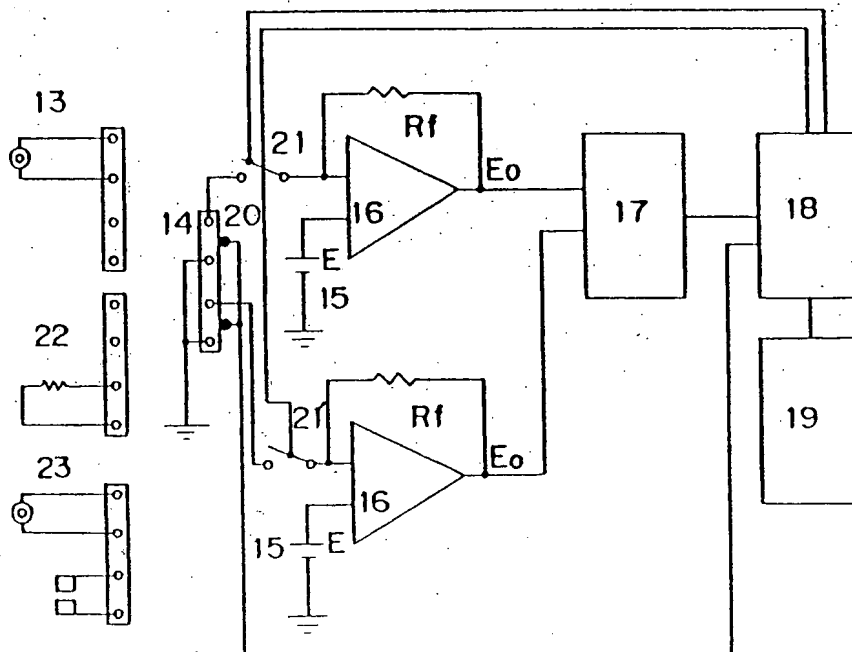


Fig. 6

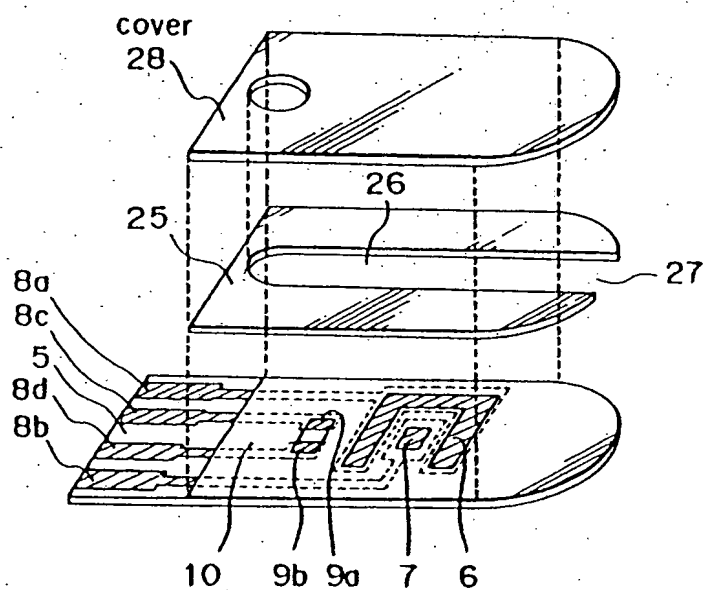


Fig. 7

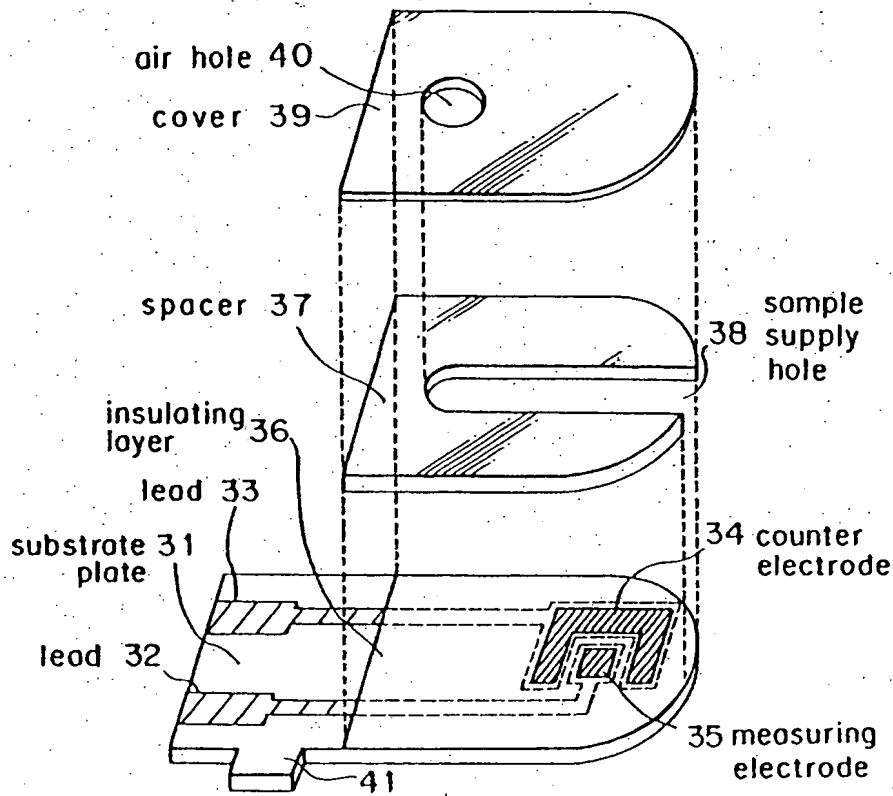


Fig. 8

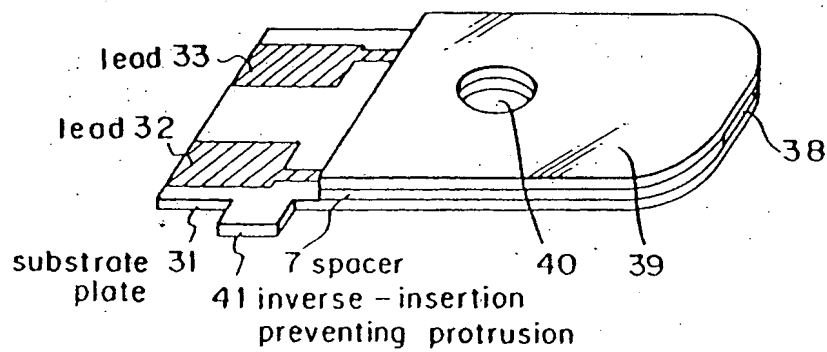


Fig. 9

